A Rapid Screening Method for Resistance to Anthonomus eugenii (Coleoptera: Curculionidae) in Capsicum (Solanaceae) spp. Plants

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Research

A rapid screening method for resistance to *Anthonomus eugenii* (Coleoptera: Curculionidae) in *Capsicum* (Solanaceae) spp. plants

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Abstract

The objectives of this study were to develop a rapid screening method for resistance to *Anthonomus eugenii* Cano (Coleoptera: Curculionidae) in pepper by assessing insect mortality and leaf consumption in seedlings as resistance traits, and analyze their interaction with leaf morphological parameters under greenhouse conditions. Seedlings were grown from seeds harvested from fruits collected from 23 populations (10 landrace and 13 wild) in Mexico as well as commercial cultivars. Leaves of 40-d-old seedlings were infested in micro-cages with 5 weevils per seedling. Eight plants were screened for each population to analyze insect mortality and leaf consumption. The experiment was replicated twice in 2 consecutive yr. The wild and landrace populations showed significantly higher number of dead adults, and lower feeding punctures and damaged leaf area from 5 to 19 d after infestation in comparison with the commercial cultivars, suggesting that wild and landrace populations are less sensitive to *A. eugenii* damage. The number of dead adults was correlated negatively with the feeding punctures and damaged leaf area, suggesting that the lower feeding damage was a result of higher *A. eugenii* mortality. This study provides a new rapid and simple method for screening resistance for control of *A. eugenii* in pepper populations and provides a promising source of resistant plant material that may be useful in breeding programs.

Key Words: antibiosis; *Capsicum annuum*; pepper weevil; plant resistance

Resumen

Los objetivos de este estudio fueron desarrollar un método de escrutinio rápido para la resistencia a *Anthonomus eugenii* Cano (Coleoptera: Curculionidae) en chiles mediante la evaluación de la mortalidad de insectos y el consumo de hojas en plántulas como caracteres de resistencia, y analizar su interacción con los parámetros morfológicos de las hojas en condiciones de invernadero. Las plántulas se cultivaron a partir de semillas extraídas de frutos recolectados de 23 poblaciones (10 variedades locales y 13 silvestres) en México, así como de cultivares comerciales. Se infestaron hojas de plántulas de 40 días en microjaulas con 5 insectos por plántula. Se seleccionaron 8 plantas para cada población para analizar la mortalidad de insectos y el consumo de hojas. El experimento se repitió dos veces en dos años consecutivos. Las poblaciones silvestres y criollas mostraron un número significativamente mayor de adultos muertos y menores pinchazos de alimentación y área foliar dañada de 5 a 19 días después de la infestación en comparación con los cultivares comerciales, lo que sugiere que las poblaciones silvestres y locales son menos sensibles al daño de *A. eugenii*. El número de adultos muertos se correlacionó negativamente con las marcas por alimentación y el área foliar dañada, lo que sugiere que el menor daño por alimentación fue el resultado de una mayor mortalidad de *A. eugenii*. Este estudio proporciona un nuevo método rápido y simple para detectar resistencia contra *A. eugenii* en poblaciones de chile y proporciona fuentes de resistencia prometedoras que pueden ser útiles en programas de mejoramiento.

Palabras Clave: antibiosis; *Capsicum annuum*; picudo del chile; resistencia de plantas

The pepper weevil, *Anthonomus eugenii* Cano (Coleoptera: Curculionidae), is a significant pest of pepper (*Capsicum annuum* L.; Solanaceae) (Addesso et al. 2014; Avendaño-Meza et al. 2015, 2016). This insect is holometabolous and oligophagous with 3 larval instars, which feed and develop completely inside the blossom buds and immature fruits of all cultivated pepper species resulting in their abscis-

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sion (Porter et al. 2007). The adult stage also causes important damage by feeding on pepper fruits, buds, flowers, and young foliage (Elmore et al. 1934; Rodríguez-Leyva et al. 2007). *Anthonomus eugenii* infestations might occur before plants reach their reproductive stage (Seal & Martin 2016). In absence of fruits, *A. eugenii* survives by feeding upon young leaves (Rodríguez-Leyva et al. 2007). *Anthonomus eugenii* early infestations on pepper crops is an important factor that may lead to great yield losses (Elmore et al. 1934; Riley & Sparks 1995). Therefore, leaf consumption is an important biological indicator to consider for the management of *A. eugenii* populations.

Anthonomus eugenii infestations are managed primarily with synthetic insecticides (Avendaño-Meza et al. 2015). The excessive use of selective synthetic insecticides for control of *A. eugenii* populations has resulted in the evolution of resistance in *A. eugenii* against various active ingredients (García-Nevárez et al. 2012; Avendaño-Meza et al. 2015, 2016). Therefore, sustainable pest management strategies must be developed. The genetic management of key pests in agriculture using resistant cultivars has been shown to be an effective and profitable control method worldwide (Seal & Martin 2016). However, no pepper cultivars resistant to *A. eugenii* are available at this time.

Before characterizing plant resistance, the first step is to select phenotypes with contrasting resistance among many accessions of wild or domesticated species using a screening method in the field, greenhouse, or laboratory. To date, few studies have accomplished the identification of sources of resistance to *A. eugenii* (Berdegue et al. 1994; Seal & Bondari 1999; Seal & Martin 2016; Rubio-Aragón et al. 2021). Previous studies by Rubio-Aragón et al. (2021) and Seal and Martin (2016) have developed screening methods on pepper fruits under laboratory and greenhouse conditions, respectively. Nevertheless, these attempts have focused on determining pepper resistance to the insect at the fruiting stage only. To the best of our knowledge, there is no information about methods for screening resistance to *A. eugenii* in seedlings or pepper leaves.

With the objective to contribute to the development of *Capsicum* spp. cultivars with resistant traits to *A. eugenii*, we propose a rapid screening method under greenhouse conditions in seedlings of wild (plants growing by themselves in an ecosystem) and landrace (domesticated local cultivar over time) pepper populations. We aim to assess *A. eugenii* mortality and leaf consumption of seedlings as resistance traits, and to analyze their relationship with leaf morphological parameters.

Materials and Methods

PLANT MATERIAL

Capsicum populations with different levels of domestication (wild and landraces) were collected from different states of Mexico. For this, mature fruits were harvested from 10 plants of 10 landrace and 13 wild populations of *Capsicum* spp. These were collected during the spring to summer season of 2016 in the southern Mexican states of Guerrero, Chiapas, Veracruz, Tabasco, Campeche, Yucatan, and Quintana Roo (Table 1). Pepper species were identified using the morphological descriptors of the International Plant Genetic Resources Institute (IPGRI 1995) for *Capsicum* species. The commercial pepper cultivars 'Fascinato' (Syngenta) and 'Maccabi' (Hazera) were used as controls due to their high susceptibility to *A. eugenii*.

COLLECTION OF *ANTHONOMUS EUGENII* ADULTS

Anthonomus eugenii infested fruits (about 2,000 fruits) from jalapeño (*Capsicum*) commercial fields were collected to carry out the experiments. The fruits were taken to the laboratory, disinfected with 0.5% bleach, washed with tap water, and dried. The clean fruits were placed in plastic containers (Especias y Plásticos Teresita, Culiacan, Sinaloa, México) and kept in a growth chamber at 28 ± 2 °C, 70 ± 5% RH, and a 12:12 h (L:D) photoperiod until the emergence of *A. eugenii* adults. *Anthonomus eugenii* identifications were confirmed using the morphological keys of Elmore et al. (1934) and Soto-Hernández et al. (2013).

Table 1. Comparison of the results of 2 consecutive experiments carried out under greenhouse conditions during spring and summer seasons of 2018 and 2019 to evaluate resistance to *Anthonomus eugenii* in pepper populations.

Experiment	Percent of dead adults	Number of feeding punctures	Visual scale of damaged leaf area
1	56.2 a	116.6 a	6.5 a
2	59.6 a	112.5 a	4.6 a

Means with different letters in columns indicate significant differences with the Mann-Whitney test ($P \leq 0.05$).

GREENHOUSE EXPERIMENT SET UP

Two consecutive greenhouse experiments were carried out to analyze leaf resistance to the *A. eugenii* in 13 wild and 10 landrace pepper populations, plus 2 commercial cultivars. Both experiments were conducted in FitoCiencia facilities located in Culiacan, Sinaloa, Mexico, during the spring and summer seasons of 2018 and 2019 with temperatures ranging from 22 °C to 32 °C inside the greenhouse.

Young pepper leaves from 13 wild and 10 landrace populations and 2 commercial cultivars were exposed for 21 d to *A. eugenii* adults. Five *A. eugenii* adults (2-d-old) were removed from the colony and confined in micro-cages fabricated manually with clear plastic cups (SOLO Cup Co., Chicago, Illinois, USA), foam sheets (La Parisina, Culiacán, Sinaloa, México), organza fabric (60 × 60 mesh, 20 × 10 caliber), and hair clips (7.3 cm diam × 4.5 cm high) with 2 detached young leaves (2–3 cm) from 40-d-old pepper plants (Fig. 1). Cotton balls were placed on the petioles of the leaves and irrigated twice daily to keep the leaves hydrated. Every assay had 25 treatments (23 populations plus 2 cultivars) with 8 replicates, and every micro-cage was considered as a replicate. Micro-cages were arranged in a completely randomized design and maintained under greenhouse conditions as described above.

RESISTANCE PARAMETERS

To study the resistance of young leaves to *A. eugenii*, the percent of dead adults, number of feeding punctures, and the visual scale of damaged leaf area were assessed as resistance parameters. For the visual scale of damaged leaf area, a scale ranging from 1 to 9 was used where: 1 = leaf with 0% of damaged area; 3 = 25% damage; 5 = 50% damage; 7 = 75% damage, and 9 = about 100% damage (Fig. 2). These data were recorded daily for 21 d after infestation. Leaf traits such as length and width (cm) were measured before the weevil infestation to determine relationships among all these parameters and resistance variables.

STATISTICAL ANALYSIS

For each of the resistance parameters, the effects of the pepper populations, the yr, and the pepper population-by-yr interaction were estimated using a 2-way analysis of variance. In all the resistance parameters and in all the 21 d analyzed in both experiments, data were not normally distributed even after transformation according to the Shapiro-Wilks test. Therefore, resistance data from both experiments were analyzed using the Friedmans non-parametric variance test and

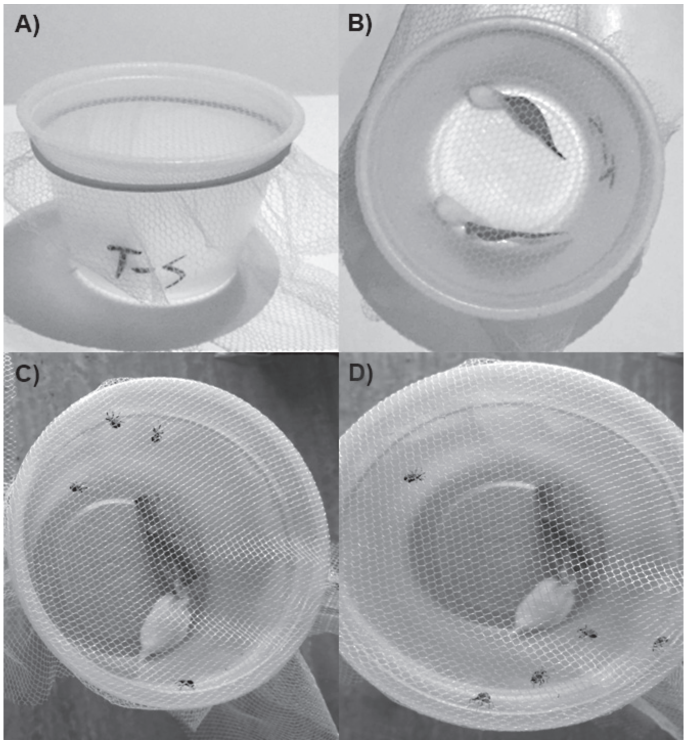

Fig. 1. Plastic micro-cages used for resistance experiments to *Anthonomus eugenii* on pepper leaves: (A) empty micro-cage, (B) micro-cage used as negative control where we placed only pepper leaves without insects, (C) micro-cage with adults of *A. eugenii* and pepper leaves, and (D) close up of 1 micro-cage with adults of *A. eugenii* and pepper leaves for resistance experiments.

the Mann-Whitney median test to determine the significance among treatments ($P \leq 0.05$) (Stell et al. 1980). Relationships between resistance parameters and leaf morphological traits were determined using the Spearman correlation test. All statistical analyses were carried out with the JMP statistical software (SAS 1995).

Results

RESISTANCE ASSESSMENT BY PEPPER POPULATION

All the resistance parameters to *A. eugenii* examined differed significantly among pepper populations but not significantly between yr ($H = 0.1164$; df = 1; $P = 0.7329$). Therefore, data obtained from both yr were pooled.

A significant difference in the number of dead adults among groups with different levels of domestication (wild, landrace, and commercial pepper populations) was observed from 5 to 19 d after infestation, whereas at 21 d after infestation all the pepper populations reached 100% dead insects ($H = 91.3051$; df = 2; $P \leq 0.001$) (Fig. 3). The landrace pepper populations showed the highest percentage mortality, followed by the wild pepper populations at 5, 7, 9, 11, and 13 d after infestation in comparison with the commercial pepper cultivars (Fig. 3). Mortality above 5% was observed in the commercial pepper cultivars at 15 d after infestation and it increased to 28, 55, and 100% at 17, 19, and 21 d after infestation, respectively (Fig. 3).

RESISTANCE ASSAYS AMONG PEPPER POPULATIONS

All pepper populations showed insect mortality, although a significant increase in mortality was observed at 13 d after infestation among pepper

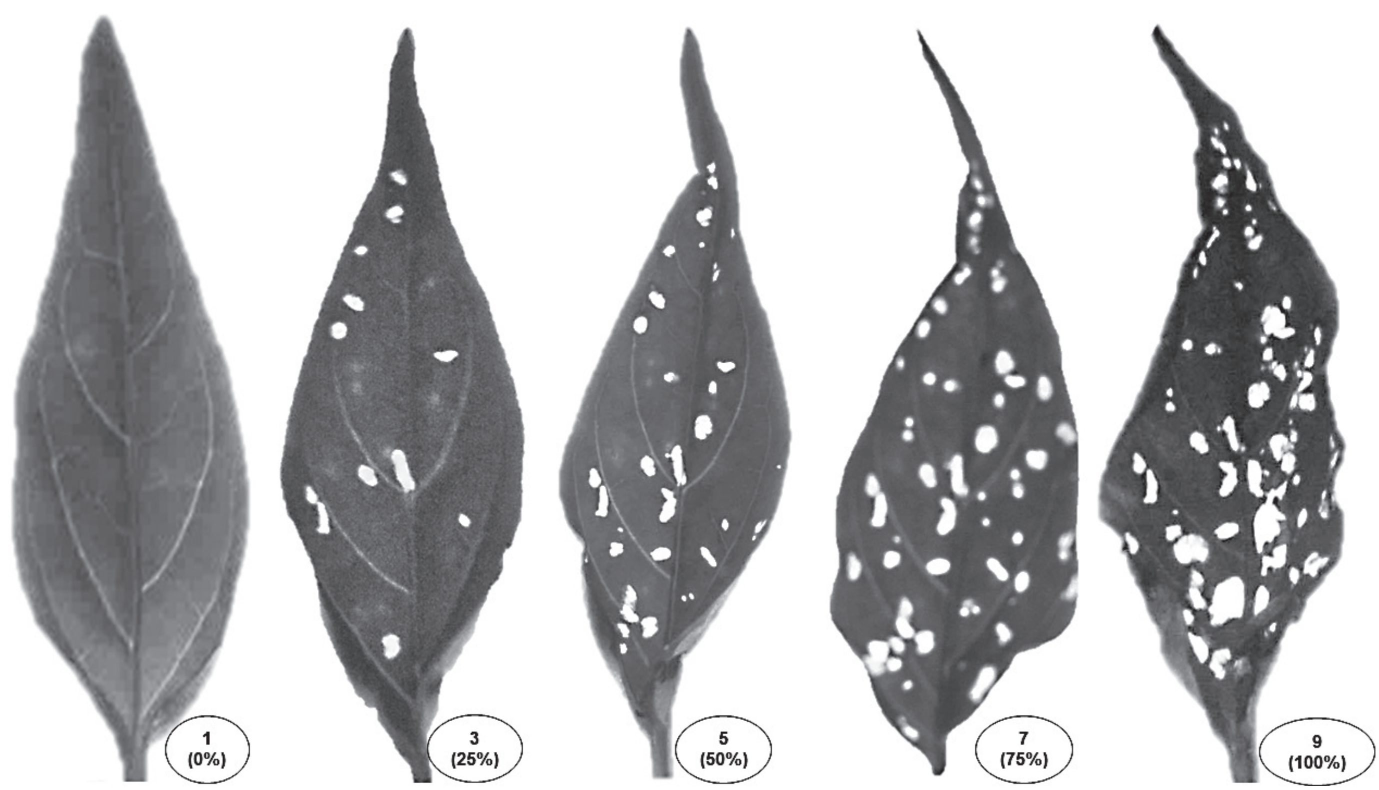

Fig. 2. Visual scale of damaged leaf area by *Anthonomus eugenii* on pepper leaves: 1 = leaf with 0% of damaged area, 3 = leaf with approximate 25% of damaged area, 5 = leaf with approximate 50% of damaged area, 7 = leaf with approximate 75% of damaged area, and 9 = leaf with approximate 100% of damaged area.

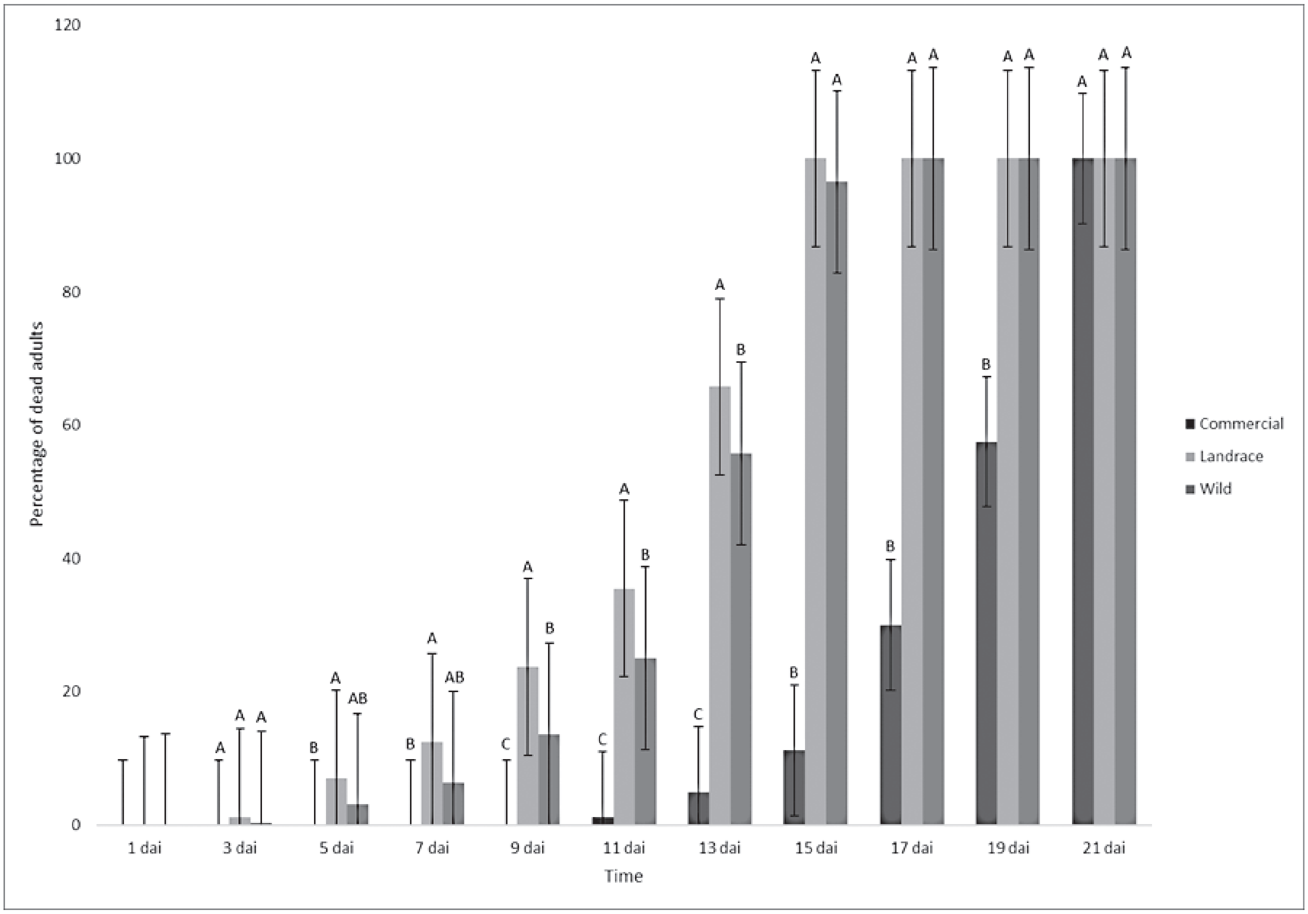

Fig. 3. Mortality (%) of *Anthonomus eugenii* adults per micro-cage during 21 consecutive d after infestation (DAI) in pepper leaves from wild and landrace populations and commercial cultivars. Bars are average percentage mortality. Comparisons made with Mann-Whitney test ($P \leq 0.05$). Different letters in the columns indicate significant differences. Error bars indicate the standard error.

populations (H = 240.1948; df = 24; $P < 0.0001$) (Table 2). All the landrace and wild pepper populations showed significant differences in percentage of dead insects in comparison with the commercial cultivars 13 d after infestation (Table 2). The levels of insect mortality were grouped into 5 categories with significantly higher percentage of dead adults in comparison with the commercial pepper cultivars (H = 132.8437; df = 4; $P < 0.0001$) (Table 2). The first with 90 to 100% of dead insects, the second with 70 to 78%, the third with 55 to 63%, the fourth with 40 to 53%, and the fifth with 25 to 30 % of dead insects in comparison with the commercial cultivars that showed only 5% of dead insects at 13 d after infestation (Table 2).

Every population evaluated had feeding damage; however, significant differences were found among populations in the number of feeding punctures, and the visual scale of damaged leaf area at 7 d after infestation (H = 357.7381; df = 24; $P < 0.0001$) (Table 2; Fig. 4). All pepper populations showed a significantly lower number of feeding punctures and visual scale of damaged leaf area with an average of 110.6 and 6.2, respectively, in comparison with the 2 susceptible commercial cultivars that had an average of 183.4 number of feeding punctures and 9.0 visual scale of damaged leaf area (H = 235.7292; df = 24; $P < 0.0001$) (Table 2).

CORRELATION BETWEEN RESISTANCE PARAMETERS AND LEAF MORPHOLOGICAL TRAITS

Significant correlations between the resistance parameters and leaf morphological parameters were detected ($P \leq 0.05$). The number of dead adults was correlated negatively with the number of feeding punctures and visual scale of damaged leaf area, and the number of feeding punctures was correlated positively with the visual scale of damaged leaf area, length, and width of the leaves (Table 3).

Discussion

A rapid screening method for resistance to *A. eugenii* in seedlings was developed. This study highlights the capacity of this method to screen for resistance to this insect in many pepper populations at the seedling stage. Other currently available methods to screen for resistance are focused on analyzing the resistance to this insect at the fruiting stage (Seal & Martin 2016; Rubio-Aragón et al. 2021), which is time consuming and tedious, thus impractical for large screening programs. In addition, the importance of *A. eugenii* leaf consumption is a biological trait that could improve agricultural management practices to reduce *A. eugenii* populations. Elmore et al. (1934) and Rodríguez-Leyva et al. (2007) reported that in the absence of plant reproductive tissues, *A. eugenii* can subsist by feeding on pepper leaves.

According to the significant lower adult mortality and higher feeding damage in the commercial pepper cultivars compared to the wild and landrace pepper populations, the methodology used to infest pepper leaves was adequate to discriminate between resistance and susceptibility to *A. eugenii*, indicating that this methodology could be useful for pepper breeding programs. This result agreed with those

Table 2. Resistance parameters for control of *Anthonomus eugenii* in 13 wild, 10 landrace, and 2 commercial *Capsicum* spp. Pepper populations are ordered by resistance level from highest to lowest.

Accession	Species	Typology	Genealogy	Origin	Percent of dead adults	Number of feeding punctures	Visual scale of damaged leaf area
UTC17	*C. annuum*	Chiltepin	Wild	Tabasco	100.0 a	92.9 lm	5.4 dg
UTC13	*C. chinense*	Habanero	Landrace	Tabasco	100.0 a	122.5 fj	6.9 bd
UTC12	*C. chinense*	Habanero	Landrace	Yucatán	100.0 a	67.0 o	4.6 fi
UTC23	*C. pubescens*	Manzano	Landrace	Veracruz	90.0 ab	105.3 im	6.0 cg
UTC21	*C. annuum*	Chiltepin	Wild	Yucatán	77.5 c	125.0 ei	6.5 be
UTC16	*C. annuum*	Chiltepin	Wild	Chiapas	72.5 cd	106.8 hm	6.0 cg
UTC01	*C. annuum*	Chiltepin	Wild	Campeche	70.0 cd	54.1 op	4.0 hi
UTC20	*C. pubescens*	Manzano	Landrace	Veracruz	70.0 cd	140.9 cf	7.9 ab
UTC06	*C. annuum*	Chiltepin	Wild	Tabasco	70.0 cd	72.1 no	4.5 gi
UTC05	*C. annuum*	Chiltepin	Wild	Chiapas	62.5 ef	143.6 ce	7.5 ac
UTC15	*C. annuum*	Chiltepin	Wild	Guerrero	57.5 fg	34.9 p	3.0 i
UTC11	*C. frutescens*	Tabasco	Landrace	Tabasco	57.5 fg	120.3 gj	6.9 bd
UTC08	*C. annuum*	Serrano	Landrace	Campeche	55.0 fg	103.0 jm	6.3 bf
UTC07	*C. annuum*	Pico Paloma	Landrace	Yucatán	52.5 gh	111.9 cg	6.9 bd
UTC04	*C. annuum*	Chiltepin	Wild	Chiapas	47.5 gh	126.0 dh	6.8 bd
UTC22	*C. annuum*	Chiltepin	Wild	Quintana Roo	47.5 gh	91.0 mn	5.4 dh
UTC18	*C. annuum*	Serrano	Landrace	Guerrero	47.5 gh	151.0 bc	7.8 ab
UTC09	*C. annuum*	Jalapeño	Landrace	Oaxaca	45.0 hi	131.8 cg	7.8 ab
UTC02	*C. annuum*	Chiltepin	Wild	Oaxaca	40.0 hi	97.6 km	5.0 eh
UTC19	*C. annuum*	Serrano	Landrace	Veracruz	40.0 hi	167.3 b	8.9 a
UTC14	*C. annuum*	Chiltepin	Wild	Oaxaca	30.0 ij	146.5 cd	7.1 bc
UTC10	*C. chinense*	Habanero	Wild	Quintana Roo	27.5 jk	115.9 gk	6.8 bd
UTC03	*C. annuum*	Chiltepin	Wild	Yucatan	25.0 k	118.0 gk	6.4 be
UTC25	*C. annuum*	Bell	Commercial	N/A	5.0 l	179.0 a	9.0 a
UTC24	*C. annuum*	Bell	Commercial	N/A	5.0 l	187.7 a	9.0 a

Accession, species, typology, genealogy, origin, number of dead adults in percentage at 13 d after infestation, number of feeding punctures, and visual scale of damaged leaf area at 7 d after infestation. Means with different letters in columns indicate significant differences with the Mann-Whitney test ($P \leq 0.05$).

reported by Porter et al. (2007) and Rubio-Aragón et al. (2021), who had similar results by infesting pepper fruits with *A. eugenii*. Additionally, the commercial pepper group began to show a significant number of dead insects from 5 d after infestation until 13 d after infestation compared to the wild and landrace pepper populations. These results indicate that the required period for evaluating mortality is from 5 to 13 d after infestation under these conditions.

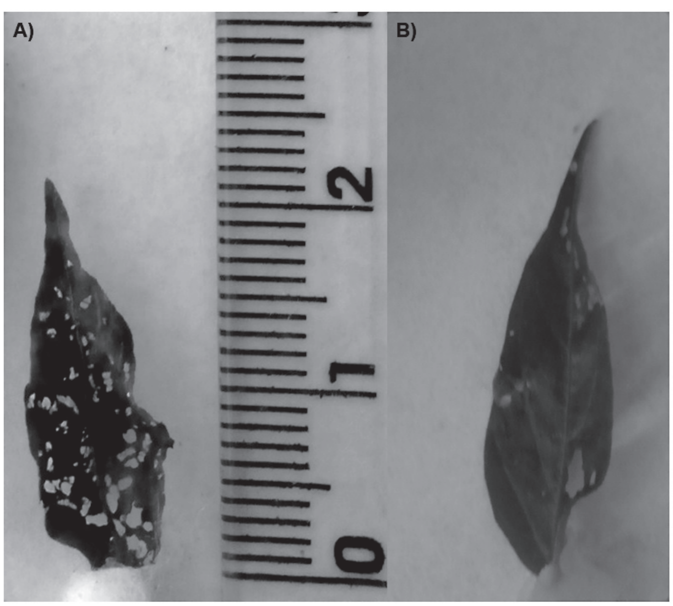

Fig. 4. Damage caused by *Anthonomus eugenii*: (A) susceptible control leaf of the Fascinato commercial cultivar with severe damage, and (B) *Capsicum annuum* plant considered resistant of the UTC17 wild pepper population collected from Tabasco, Mexico, infested with *A. eugenii*. Picture was taken 7 d after infestation.

This research showed that wild and landrace pepper populations had a significantly higher *A. eugenii* mortality and lower feeding damage in comparison to the commercial group during 2 independent experiments. These results indicate that the broad genetic variability of pepper populations in Mexico is an important resource to combat *A. eugenii*, and such genetic diversity could support breeding programs by improving multiple resistance traits to this insect, such as leaf-feeding mortality in modern pepper cultivars. Our results agreed with those of Latournerie-Moreno et al. (2015) and Rubio-Aragón et al. (2021), who reported that wild and landrace pepper populations from Mexico are valuable sources of resistance to key pepper pests such as whitefly and *A. eugenii*. Additionally, these results indicate that wild and landrace pepper populations differ in their level of *A. eugenii* resistance, and are more resistant than cultivated varieties of pepper under no-choice resistance tests. These results support previous studies indicating that cultivated species are less resistant to herbivores than wild and landrace populations (Garzón-Tiznado et al. 2020; Millán-Chaidez et al. 2020).

Several wild and landrace pepper populations showed a significant percentage of dead adults and lower leaf-feeding damage in comparison to the commercial pepper cultivars, suggesting that different levels of resistance and antibiosis mechanisms might be involved. Another explanation of the high insect mortality in these wild and landrace pepper populations could be attributed to the unsuitability of these leaves regarding their hardness, thickness, pubescence, and chemical compounds, which can harm the insect, repel it, or complicate its feeding.

Table 3. Relationships between resistance parameters and leaf morphological traits.

	Number of dead adults	Number of feeding punctures	Visual scale of damaged leaf area	Length of leaves
Number of feeding punctures	−0.299***			
Visual scale of damaged leaf area	−0.310***	0.864***		
Length of the leaves	−0.027	0.236**	0.018	
Width of the leaves	−0.117	0.195*	−0.019	0.627***

Correlations made with Spearman test. *Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level. ***Correlation is significant at the 0.001 level.

Because it is known that *A. eugenii* are long-lived and have the ability to survive an average of 78.7 d under controlled conditions (Addesso 2007), we suggest that the mortality of this insect on these populations are more related to an antibiosis or antixenosis mechanism. However, further studies on all these topics must be carried out before conclusions can be made. Our results agreed with those of Rubio-Aragón et al. (2021), who found pepper fruits of different populations showing antibiosis mechanisms by increasing *A. eugenii* mortality and reducing fruit-feeding damage. These results indicate that this screening method allowed discrimination among different levels of resistance or host suitability to this insect, and demonstrated that just as pepper fruits can be used to test for resistance to *A. eugenii*, pepper leaves also can be used for the same purpose because both play an important role in the biology of the insect. These results also indicate that the leaves of the pepper populations with significantly higher mortality rates possess defense mechanisms that are harmful to the adults of *A. eugenii,* or that these plants are less suitable to this insect. Host plant quality is a key factor that affects the antibiosis response of plants to insects (Mottaghinia et al. 2011). The ability of pepper survival to major pests such *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) and *Aphis gossypi* Glover (Hemiptera: Aphididae) is influenced by different biomolecules such as enzymes, amino acids, and secondary metabolites of the host plants (Latournerie-Moreno et al. 2015; Daryanto et al. 2017; Jeevanandham et al. 2018). Further studies to identify the biomolecules involved in *A. eugenii* resistance will be needed. To the best of our knowledge, this is the first report of resistance to *A. eugenii* in pepper leaves.

Wild and landrace populations of *Capsicum* spp. have been in contact with *A. eugenii* in the same geographic area as the cultivated pepper in Mexico, at least during the last century (Avendaño-Meza et al. 2015, 2016); therefore, these populations might have been exposed to some extent to the selective pressure imposed by *A. eugenii*, which is native to Mexico. This constitutes a plausible explanation for the different resistance levels to *A. eugenii* detected in the wild and landrace pepper populations evaluated here. At the same time, wild and landrace populations of pepper have not been sufficiently explored as a source of genetic resistance to *A. eugenii* (Retes-Manjarrez et al. 2018). For this reason, these resources must be incorporated in future breeding programs to increase pest resistance in pepper, and to bring new variability that could contribute to improve other agronomical traits such as yield, vigor, color, and flavor. Wild and landrace relatives of cultivated plants are an important genetic resource that constitutes a primary gene pool that can help to solve problems in today's agriculture, such as resistance to pests and diseases, and to increase the quality and quantity of production (Hernández-Verdugo et al. 1998).

On the other hand, some of the pepper populations (i.e., UTC04, UTC03, UTC22, UTC02, and UTC14) that showed resistance to *A. eugenii* in pepper fruits from the previous study of Rubio-Aragón et al. (2021) were susceptible in this study, and 11 (i.e., UTC15, UTC06, UTC23, UTC12, UTC16, UTC01, UTC17, UTC08, UTC21, UTC05, and UTC13) showed resistance to *A. eugenii* under the leaf-feeding assay. These results indicate that the resistant traits of fruits and leaves could be regulated or governed by different genes. These results are similar to those reported by Sy et al. (2005) who reported that the genes that conferred resistance to *Phytophthora capsici* Leonian (Peronosporales: Peronosporaceae) in the roots are different from those that conferred resistance to this pathogen in the stem and in the leaves of the same genotype. Breeding programs must take into account that the response of *A. eugenii* to leaves is different from the response of this insect to the fruits of these pepper populations with different levels of resistance.

The interactions among the number of dead adults, number of feeding punctures, and visual scale of damaged leaf area indicate that populations with high *A. eugenii* mortality have less damage by this insect. This agrees with Rubio-Aragón et al. (2021), who found that populations with less fruit damage by weevils had a larger number of dead adults of *A. eugenii*. The number of feeding punctures was correlated significantly and positively with the visual scale of damaged leaf area, indicating that this scale is a valuable tool that can be used to determine the foliage consumption by *A. eugenii* in a precise way. This resistance parameter also correlated with the width and length of the leaves suggesting that *A. eugenii* feeds more in leaves with higher biomass. A plausible explanation for this interaction is that commercial cultivars have better desirable agronomic traits, such as bigger leaves. This result agreed with Rubio-Aragón et al. (2021), who detected a similar pattern in fruit infestations.

The broad genetic variation exhibited by the wild and landrace pepper populations from Mexico allowed the detection of plants with resistance mechanisms in their leaves to *A. eugenii*; therefore, such variability must be studied and preserved. The resistant populations detected in this research could be a promising source of resistant plant material that could be used to support breeding programs to develop pepper cultivars with multiple resistant traits to this insect.

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